LARGE DEFORMATION STRESS RELAXATION AND BI-AXIAL COMPRESSION RECOVERY OF GLUTEN REPRESENTING DIFFERENT WHEAT CLASSES

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ABSTRACT

Despite the great variety of physicochemical and rheological tests available for measuring wheat flour, dough and gluten quality, the US wheat classification system still relies primarily on wheat kernel hardness and growing season to differentiate between cultivars. To better understand and differentiate wheat cultivars of the same class, the tensile strength, and stress-relaxation behavior of gluten from 36 wheat cultivars was measured and compared to other available physicochemical parameters, including but not limited to protein content, glutenin macropolymer content (GMP) and bread loaf volume . In addition a novel compression-recovery (CORE) instrument was used to measure the degree of recovery of gluten from 15 common US wheat cultivars. Gluten tensile strength ranged from 0.04 to 0.43 N at 500% extension, while the degree of recovery ranged from 5 to 78 %. Measuring gluten strength clearly differentiates cultivars within a wheat class; nonetheless it is not a good predictor of baking quality on its own in terms of bread volume. Gluten strength is highly correlated with mixograph mixing times (r=0.879) and degree of recovery (r=0.855), suggesting that dough development time is influenced by gluten strength and that the CORE instrument is a suitable alternative to tensile testing, since it is less time intensive and laborious to use.

BIOGRAPHICAL SKETCH

Stephen Josef Chapman was born in England, grew up in Germany, and went to school in the United States. He hopes to graduate with his Masters in Food Science in May 2011 from Cornell University.

The Purple Giant

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LIST OF ABBREVIATIONS

AACC	American Association of Cereal Chemists
BLV	Bread loaf volume
DE	Degree of elasticity measured by the TA-XT_{plus}, defined as the ratio of un-dissipated equilibrium force over the maximum force of extension
DR	Degree of recovery measured by the CORE, defined as the ratio of overall distance recovered over the distance compressed
GIPSA	Grain Inspection, Packers and Stockyards Administration
GMP	Glutenin macropolymer
HMW-GS	High molecular weight-glutenin subunits
HDWH	Hard white
HRW	Hard red winter
HRS	Hard red spring
SWH	Soft white
ZSV	Zeleny sedimentation volume

LIST OF SYMBOLS

F_{max}	Maximum force measured at specified extension
F_{equi}	Equilibrium force measured at specified extension

CHAPTER 1

BACKGROUND

1.1 Wheat Constituents and their Relevance to Dough and Bread Making

Wheat (*Triticum*) is a grass that originated in the Fertile Cresent region of eastern Asia, but is cultivated worldwide, and is the third most produced cereal after maize and rice and the greatest source of vegetable protein worldwide (Food and Agriculture Organization of the United Nations., 1997). The kernel of the common wheat or bread wheat (*Triticum aestivum*) consists of three main components: the bran, endosperm and embryo also referred to as germ (Fig. 1). As a whole an average wheat kernel consists of 14% water, 12% protein, 60% starch, 10% fiber, 2% lipids and 2% minerals, although there can be significant variations between wheat cultivars (Sluimer, 2005). The endosperm represents about 85% of the wheat kernel by weight, and contains the majority of starch and protein, although not the highest concentration of protein (Sluimer, 2005). The endosperm provides the nutrients necessary for the developing plant. The bran, which makes up 12% of the wheat kernel by weight, consists of the aleurone and pericarp region, which form the hard outer layer of the kernel and are rich in fiber, proteins, vitamins and minerals (ash) (Sluimer, 2005). The germ is the embryo of the seed and contains the reproductive parts that can eventually germinate into a plant. The germ accounts for 2% of the kernel by weight, and has the greatest concentration of lipids at ~ 12% (Sluimer, 2005).

During milling the outer layers are removed from the endosperm, which is reduced in size to yield flour sized particles (MacRitchie, 2010). Generally white flours only consist of the endosperm layer of the wheat kernel, whereas whole wheat flours contain both the bran and endosperm layers and are considered to be a healthier alternative based on the greater amounts of fiber and minerals.

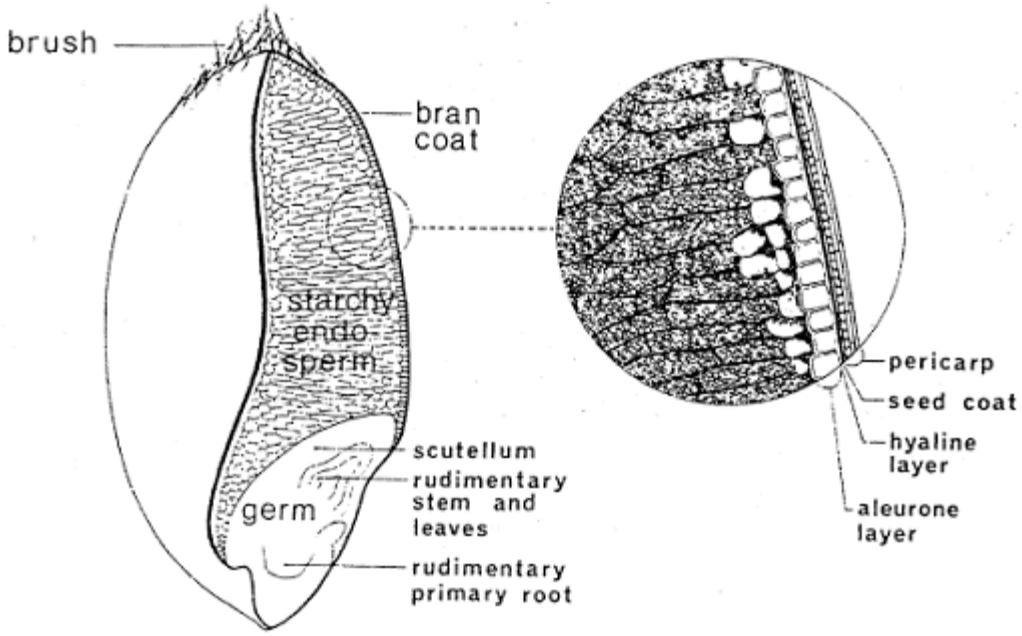

Figure 1 Cross-sectional view of a wheat kernel and its constituent parts (Anonymous 2000)

1.1.1 Starch

The largest component of wheat flour is starch, which primarily consists of amylose and amylopectins. Both consist of glucose subunits, but amylose has a linear arrangement and can have a molecular weight of up to 1 thousand kDa, while amylopectins are highly branched and one of the largest biopolymers known at a molecular weight of 1 million kDa (Sluimer, 2005). These large sugars form lenticular (A granules) and polyhedral (B granules) starch granules, which differ significantly in size (Sliwiniski, 2003). These starch granules have both amorphous and crystalline regions, where the crystallinity causes birefringence and x-ray diffraction (Sliwiniski, 2003).

At room temperature the starch granules are very hard; barely absorb any water beyond 40% of their weight and are resistant to most enzymatic action. Only once greater temperatures in the range of 53 – 63 ºC are reached, do the starch granules absorb significant amounts of water and swell, thereby disrupting the crystalline regions and the overall granule structure (Sluimer, 2005). This allows amylose to leak into the surrounding medium and gelatinize. At these temperatures wheat amylases break down some of the starch molecules, which have beneficial effects on ovenspring and the softness of the bread crumb (Sluimer, 2005).

On the other hand, as a result of milling, damaged starch granules absorb water and swell at room temperature, and are much more susceptible to enzymatic breakdown. Maltose produced by the breakdown of amylose can be used directly by yeast to make carbon dioxide for bread leavening. Therefore some degree of granule damage is desirable, providing yeasts which act as leavening agents enough substrate to produce carbon dioxide. Nonetheless too much starch breakdown leads to a sticky, difficult to handle dough (Sluimer, 2005).

1.1.2 Non-Starch polysaccharides

A small portion of wheat kernel consists of cellulose, hemicelluloses and pentosans, which are not commonly digestible by the human gut. These nondigestible sugars are also called fiber. These fibers are commonly found in the cell walls, and are most abundant in the bran fraction of flour. As fibers can absorb 10 times their weight in water, they tend to have a somewhat detrimental effect on whole wheat breads, since they have far greater bran content than breads made out of white flours (Michniewicz et al., 1992).

1.1.3 Proteins

Originally wheat kernel protein fractions were classified by their relative solubility based on a method developed by Osborne (Osborne, 1907) into five different categories. The first consists of albumins which are water soluble, globulins which are soluble in salt solutions, gliadins which are soluble in 70% ethanol and glutenins which are soluble in acidic or basic solutions. Nonetheless there is a completely insoluble fraction, which is considered to be part of the glutenin fraction (Sliwiniski, 2003). The albumins and globulins are largely enzymes, including amylases, proteases, lipases, lipoxygenases and phosphatases (Sliwiniski, 2003). The glutenin and gliadin fractions (gluten) will be discussed in greater detail further on. Despite thoroughly washing flour with 2% salt water, the remaining glutenin and gliadin fraction still contains significant quantities of starch and lipids (Roels, 1997).

1.1.4 Lipids

Whole wheat flours have about 2% fat, while white flours less than 1%. The fats are mostly polyunsaturated linoleic acid, and are believed to play a role in oxygen uptake during mixing (Sluimer, 2005). On the whole their role in the bread making process is still understood poorly, although they have significant detrimental effects on the shelf life of flours, which can become rancid with time.

1.1.5 Minerals

The mineral content or ash percentage of the wheat kernel is 8% overall, where the aleurone layer consists of 15% ash, while the wheat endosperm has only about 0.4% ash by weight (Sluimer, 2005). Ash levels by themselves do not have an effect on bread quality, nonetheless higher ash levels go hand in hand with greater germ and bran content.

1.2 Gluten Structure & Function

A large body of work has been published using a wide range of rheological methods on both dough and gluten, in combination with various other analytical techniques to unlock the unique functional properties of wheat which allow for the elastic, air entrapping nature of wheat dough. The ability of dough, which primarily consists of wheat flour and water, to entrap gas during proofing and baking has been linked to the viscoelastic properties of gluten, the main proteins in wheat (Schofield & Blair, 1932). These proteins are divided into glutenins and gliadins, based on their relative solubility in ethanol (Osborne, 1907), although structurally they are closely related (Shewry & Tatham, 1990). Gliadins are single-chain polypeptides which primarily form intramolecular disulphide bonds and can range in molecular weight (MW) from 2×10^4 to 7×10^4, while glutenins are multiple-chain polymeric proteins whose subunits are linked via a network of intermolecular disulphide and hydrogen bonds, and can range in MW from 10^5 to over 10^8 (Weegels et al., 1996). During dough mixing this protein network is further developed and dispersed throughout the starch water system. See Fig. 2 for an E-SEM image of an optimally developed dough showing starch granules dispersed on aggregated protein strands.

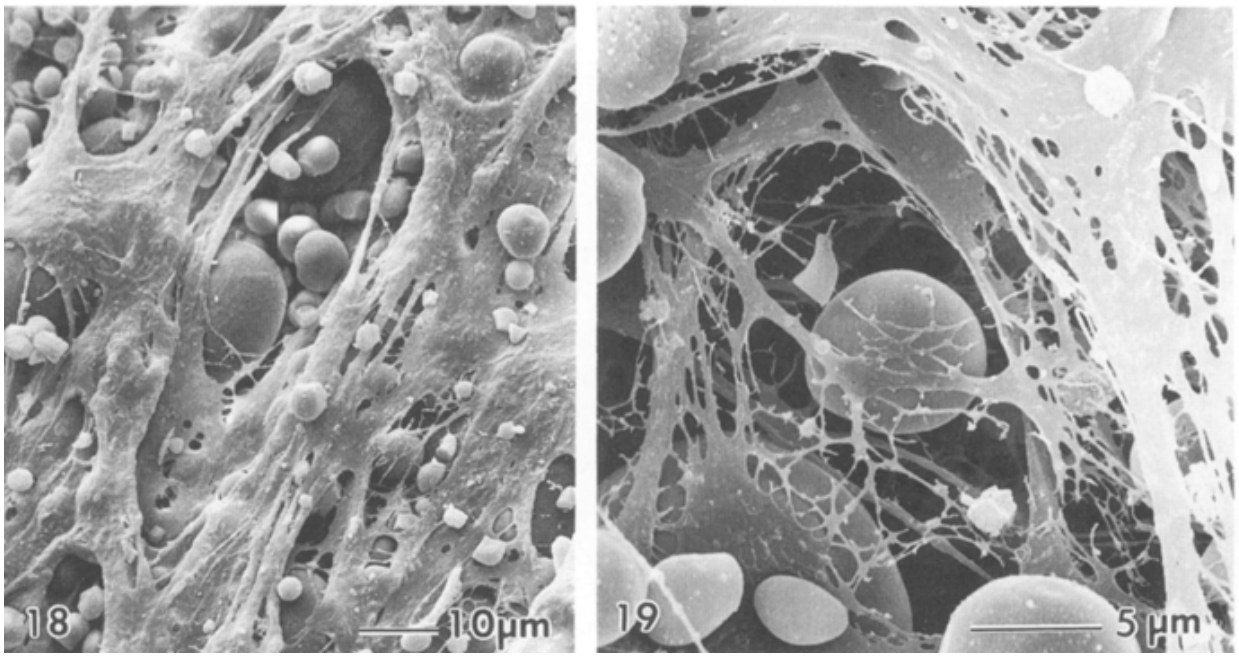

Figure 2 E-SEM image of optimally developed wheat dough (Amend & Belitz, 1990)

If maximizing bread loaf volume is the ultimate criterion for wheat bread making quality, then a high concentration of gluten protein is a good indicator. Nonetheless, protein quantity in itself does not explain all variation in bread loaf volume (Finney & Barmore, 1948). On the one hand the gliadin fraction is considered responsible for the viscous flow component in gluten (Shewry PR et al., 2003), and on the other hand varying amounts of different types of high and low molecular weight (HMW/LMW) glutenin subunits (GS), which are essential for forming an elastic polymer network, have been shown to have different "qualitative" effects on bread making (Shewry et al., 1992).

These HMW-GS are rich in glutamine, glycine and proline, consisting of non-repetitive C and N terminal domains which primarily form alpha helices, enclosing long central repetitive domains (Shewry & Tatham, 1990). The central domains of y-type subunits consist of hexapeptide (PGQGQQ) and nonapeptide repeats (GYYPTSLQQ), while the x-type subunits have hexa- (PGQGQQ), nona - (GYYPTSPQQ) and tripeptide repeats (GQQ) (Shewry & Tatham, 1990, Shewry et al., 1992, Lindsay & Skerritt, 1999). As a result of the high abundance of proline and glutamine amino acids the center repetitive domains of the glutamine units are believed to consist largely of beta-turns and beta-sheets (Tatham et al., 1985), (Sliwinski et al., 2004).

There are several models that attempt to explain some of the structure function relationships between gluten subunits. The first model proposed by Ewart suggests that glutenin consists of individual subunits bound by covalent disulfide bonds into linear polymers (Ewart, 1972, Ewart, 1968, Ewart, 1979). In this hypothesis the viscous flow is caused by molecular slippage and disulphide bond interchange, whereas elasticity is determined by the cross linked subunits. Extensions to this linear

concept of gluten elasticity involves the belief that the presence of cysteine residues at both N- and C- terminal domains of some gluten subunits would allow for more extensive branching and cross-linking. This branched polymer concept was further evolved into a point entanglement model (Damodaran & Paraf, 1997) where small areas of the polymers interact beyond just disulphide bridges. Nonetheless the most cogent explanation for the elastic behavior of gluten is put forward by Belton, who explains the elastic, restoring force in terms of enthalpic and entropic changes upon stretching (Belton, 1999). In simple terms the restoring force upon gluten extension comes from the change in entropy of the system, going to a more ordered state upon stretching.

1.3 Wheat quality testing

Many different tests designed to assess and predict the quality and characteristics of wheat exist. These range from baking tests, rheological dough and gluten tests to chemical analyses, all of which may or may not be used depending on the desired information and end use.

1.3.1 Baking tests

Baking tests generally follow standardized methods for the quantity and type of flour, amounts of yeast, salt, water and other optional ingredients, and are often used to give subjective insight into the type of crust, overall appearance, texture, softness and the more objective measurement of loaf volume. The overall loaf volume is often considered one of the primary quality indices of wheat flours. The method for assessing bread loaf volume used here for instance follows method 10-10B from the American Association of Cereal Chemists (AACC International., 2009).

1.3.2 Rheological tests

Rheology measures the deformation (strain) of a material in response to the application of a mechanical force (stress). There different ways in which stresses are

applied, the three main ways being extension, compression and shear stresses (See Fig. 3). Furthermore these rheological tests are further differentiated based on whether they are fundamental or empirical tests, and whether they are large or small deformation tests. Dobraszczyk and Morgenstern review many of the rheological tests available for cereal products (Dobraszczyk & Morgenstern, 2003). Empirical tests are descriptive in nature and are not suited for reporting any fundamental rheological parameters such as stress, strain, strain rate, modulus or viscosity and most cases the measurements are greatly dependent on the sample size and shape, making it challenging to compare results between different instruments or extrapolate to relevant processing conditions. Small deformation tests are primarily shear oscillation or shear creep tests, while large deformation tests (strains > 5%) are mostly extension and creep tests. Large deformation tests for dough systems are generally more useful since they are more relevant to dough processing conditions. For instance the strain in gas cells during proofing is in the range of several hundred percent (Amemiya & Menjivar, 1992), while mixing involves a series of large deformation dough stretching actions. As a result most small deformation shear rheology tests are not applicable to large deformation processing conditions, since these tests are also conducted in a narrow frequency band in the plateau region of the materials, which have been shown to be independent of the polymer MW (Ferry, 1980).

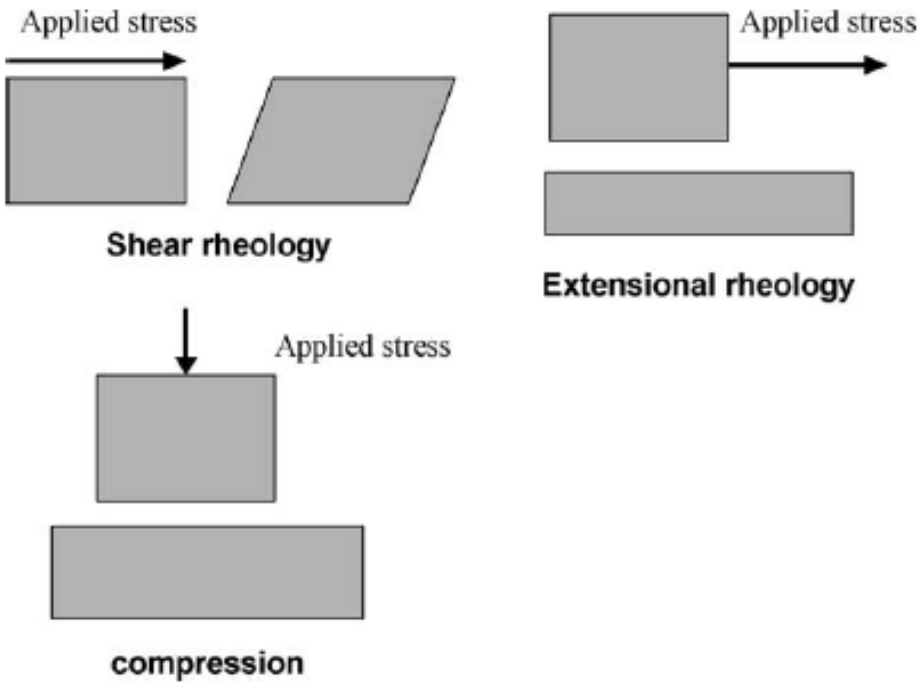

Figure 3 The three main ways of measuring deformation after mechanical stress application (Belton, 2005)

There exist many dough tests that try to assess the suitability of flour dough's for different bread making applications. These are mostly empirical measurements of parameters such as dough extensibility, development time and stability and can be used to assess proper dough/gluten network development, water absorption and even have some correlation with bread loaf volume. The most common rheological tests include the farinograph dough mixer, the extensigraph and the alveograph.

The farinograph dough mixer measures the torque or force applied by the dough against two mixing blades in a double walled mixing chamber as a function of time. The temperature of the dough can be controlled by flooding the chamber walls with thermostated water, while the blades knead at a constant speed. This test follows AACC Approved Method 54-21 and allows four parameters to be measured (AACC International., 2009). These include i) dough development time, which measures the time in minutes till the maximum resistance of the dough is reached during mixing, ii) the dough stability time, which measures the residence time of the dough above a resistance of 500 brabender units, iii) the degree of softening, which is the difference in resistance between the peak and that measured 12 minutes afterwards, and iv) the overall energy requirement needed for dough mixing, which corresponds to the area under the curve.

The extensigraph stretches equal sized dough cylinders to the breaking point at constant speed, while measuring the force of resistance as a function of time. This test follows AACC Approved Method 54-10 and measures three parameters (AACC International., 2009). These include the resistance to stretching, maximum resistance (R_{max}) and overall extensibility. See Fig. 4 for a picture of a piece of dough being stretched on a Brabender extensigraph, and the resultant force measured as a function of extension.

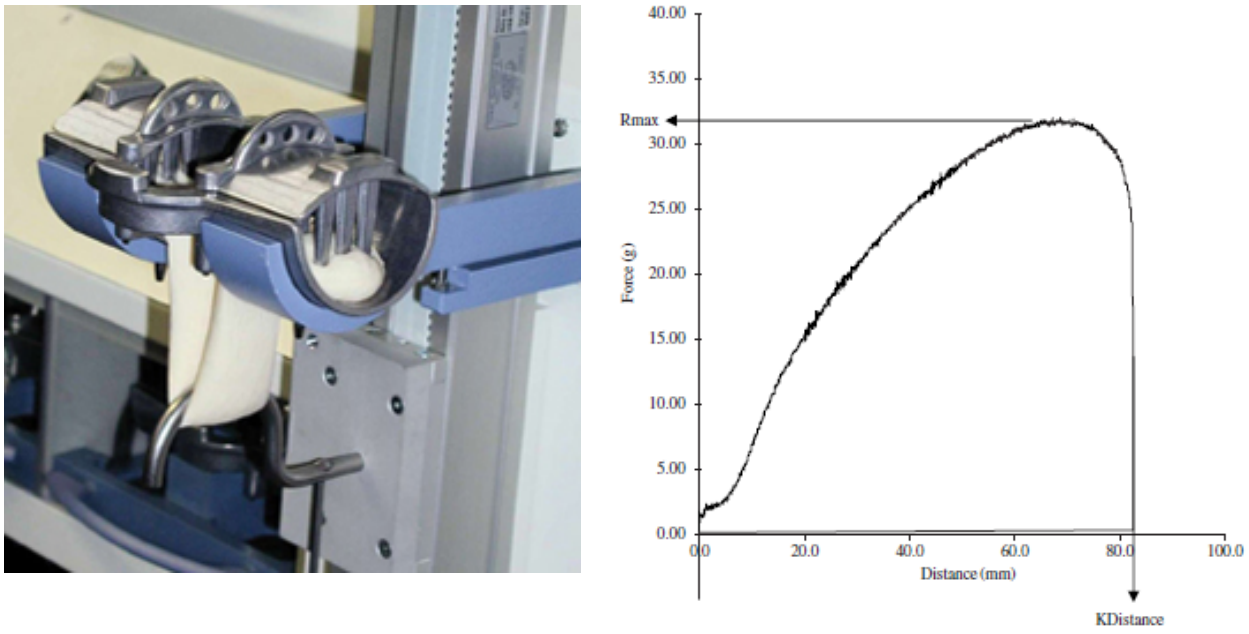

Figure 4 A piece of dough being stretched by a hook on a Brabender extensigraph, and the resulting force measurements. ("Extensigraph" – May 11th, 2011 <http://www.cwbrabender.com/ExtensographE.html>)

The alveograph is a bi-axial variant of the extensigraph, which measures the change in air pressure used to form an air pocket in a sheet of dough over time. This test follows AACC Approved Method 54-30A, and measures three parameters (AACC International., 2009). These include the maximum resistance of the dough bubble, the maximum size of the bubble, and the energy of deformation. The principle of the alveograph is shown in the figure below (Fig. 5), along with an example of data output.

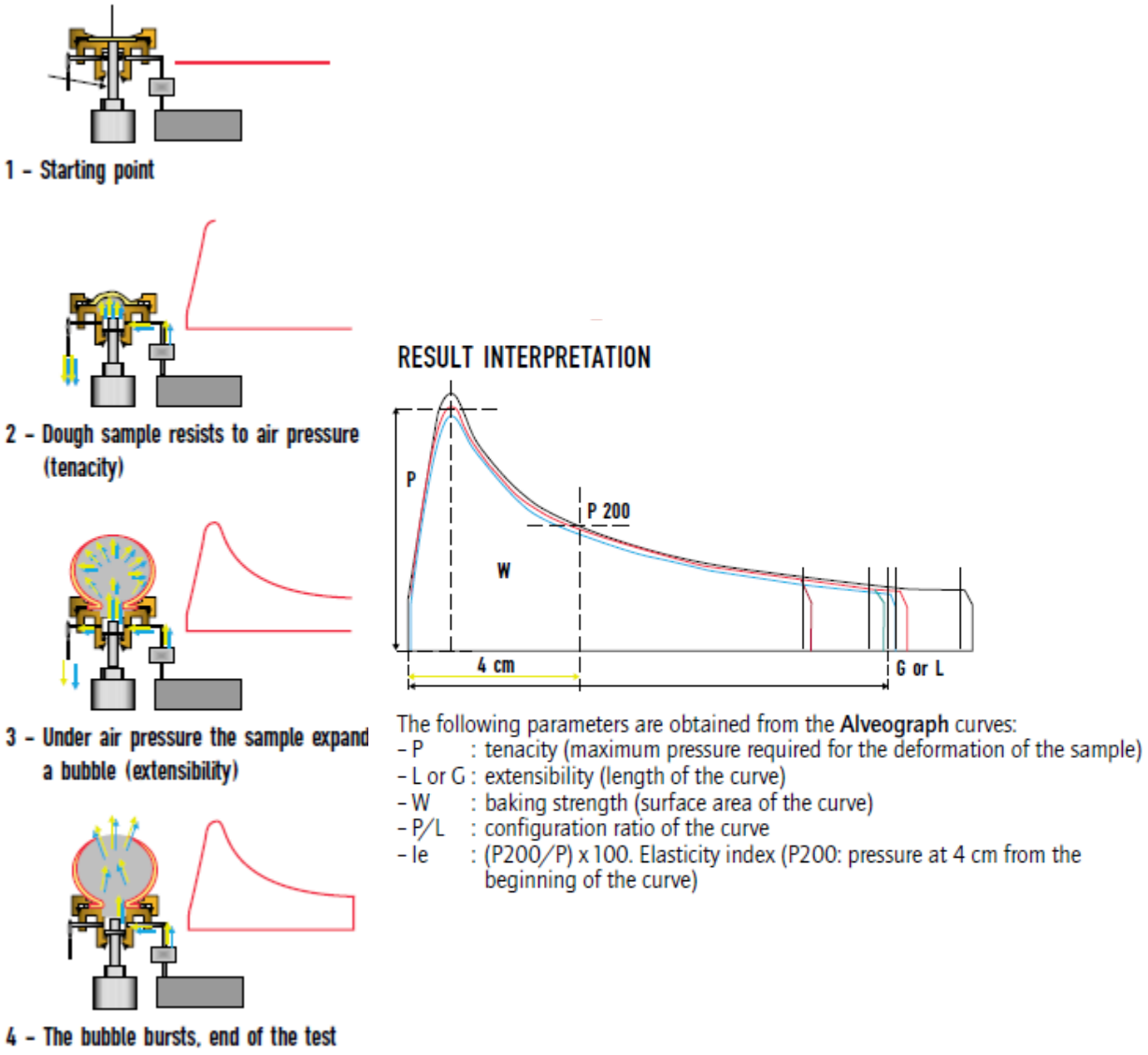

Figure 5 Principle of the alveograph dough bubble tester.

("Alveograph NG – Standard Methods" - May 11th, 2011 <http://www.agrionica.hr/_leafleats/alveograph.pdf?lang=en>)

Most of these physical dough tests measure variants of the same intrinsic quality of dough in different ways. Due to their empirical nature they do not allow for easy standardization, are only useful for specific applications and are not too useful for gaining insight into the more fundamental aspects of wheat quality and functionality. Nonetheless some large deformation rheological approaches have also been applied to purified wheat gluten. Similar to the extensigraph, Zhao et al (2010) have adopted a TA-XT_{plus} texture analyzer (Texture Technologies, Scarsdale, NY) to conduct tensile and extension measurements on gluten from various cultivars. Fig. 6 shows a Texture Technologies TA-XT2, an older model of the TA-XT_{plus} with a 5 kg load cell, which can be used for a variety of tensile and compression tests, along with a variety of load arm attachments.

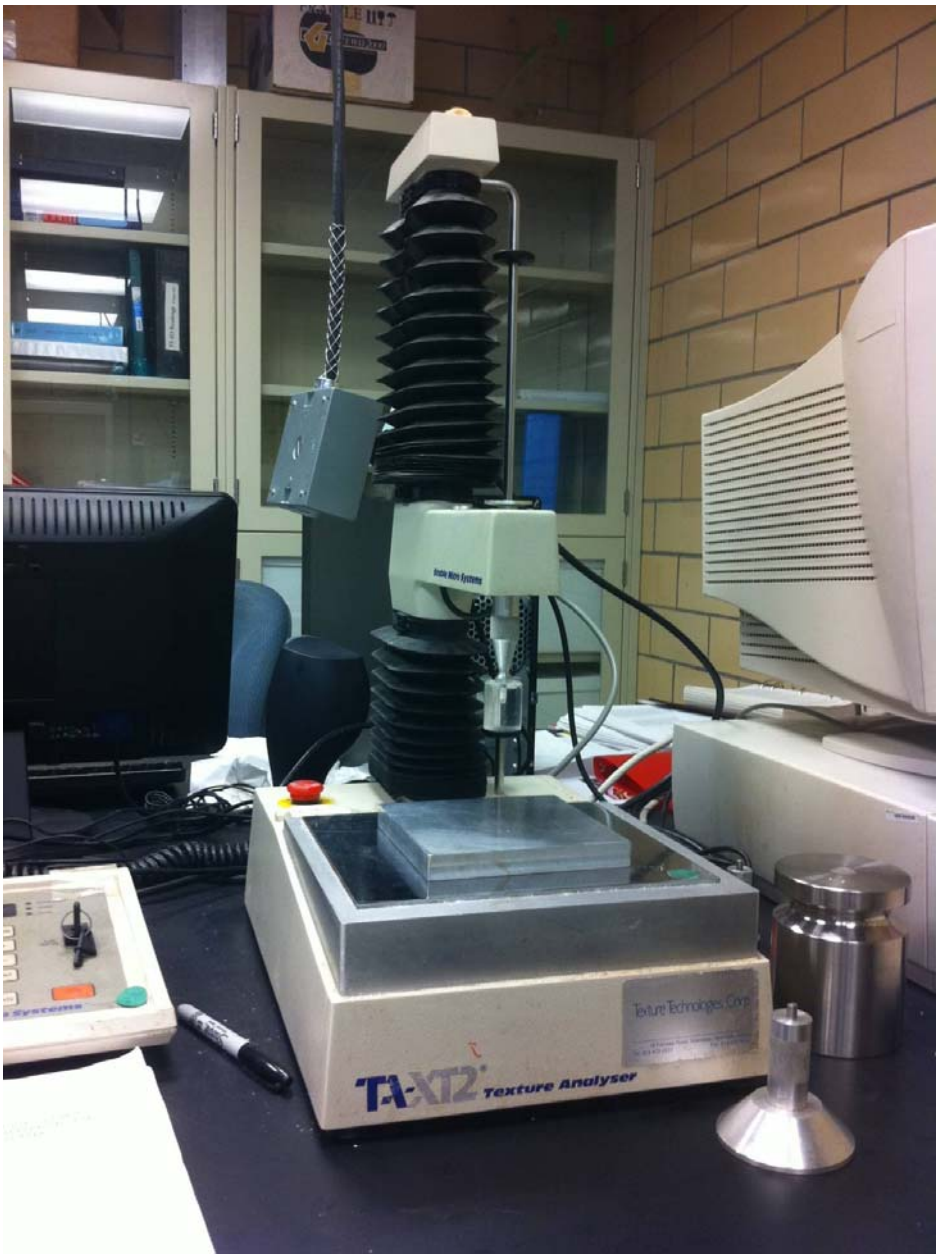

Figure 6 Texture Technology TA-XT2 with a 5 kg load cell.

1.3.3 Chemical analysis

Since wheat flour has quite variable moisture content, the chemical analysis data for flour can be reported in several ways: on a 14% moisture basis, where all results are adjusted to the assumption that 14% of the weight is due to water; on a dry matter basis, where all values are adjusted by removing the water fraction, which leads to higher percentage values; and on an as-is basis, which is mostly used by nutritionists (Sluimer, 2005).

The moisture content of flour is obtained by measuring the change in mass of a sample before and after drying in an oven set at a specific temperature over a defined time period (AACC Approved Method 44-15A/44-16 - (AACC International., 2009)). The water content of flour influences its water activity and a water activity above 0.8 can lead to the growth of molds, thus shortening its shelf life significantly if not stored in an appropriate way.

The protein content of flour is determined by measuring the nitrogen content (Kjeldahl method/AACC Approved Method 46-10 - (AACC International., 2009)), although IR spectroscopy is increasingly being used as well. Another empirical method for estimating the protein content and quality of wheat flours is the Zeleny sedimentation value (AACC Approved Method 56-61A), where the flour is shaken in an isopropyl alcohol solution and allowed to settle (AACC International., 2009). The height of the settled flour after a certain time period is given as the sedimentation value.

Other chemical measurements include ash content and falling number measurements. According to AACC Approved Method 08-01 a flour sample is incinerated until a constant weight remains, which is indicative of the flour mineral content. The falling number on the other hand measures the alpha – amylase activity

of flour (AACC Approved Method 56-81B - (AACC International., 2009)). Flours with sprout damage tend to have a lower falling number, which can be adjusted by adding in diastatic malt flour (Sluimer, 2005).

1.4 Objectives

In 2003 GIPSA conducted an ideation meeting and review of U.S. Wheat Associates global wheat testing methods to assess opportunities for improving wheat quality testing methodologies and instruments. GIPSA's survey indicated a need for easier and more rapid dough and gluten strength tests (Chinnaswamy et. al., 2005), which would provide additional wheat quality parameters useful for distinguishing wheat cultivars beyond available rheological and physicochemical analyses for wheat breeders, wheat regulatory agencies and others involved in wheat grain trade. This thesis presents part of an effort to develop a more rapid gluten strength test, and gain further insight into the behavior of gluten and dough under different strains.

This thesis has several main objectives, which will be discussed and related in three separate chapters. They are as follows:

1) To determine the large-deformation stress relaxation behavior of gluten representing a set of 36 wheat cultivars from several different wheat classes under different strains

2) To determine the bi-axial compression recovery behavior of gluten from a subset of 15 wheat cultivars representing different wheat classes with a newly developed prototype CORE instrument built by Perten Instruments

3) To correlate the different rheological parameters measured with the large deformation stress-relaxation and compression-recovery tests with other available wheat quality tests

4) To investigate the possibility of using a similar stress-relaxation test for measuring dough strength, and the relationship between dough and gluten strength under similar strain conditions

This work is expected to provide a novel and improved system for measuring dough and gluten strength, and present insight on the relevance of gluten strength in relation to other wheat quality parameters. In addition this thesis will highlight several interesting observations made during the investigation.

CHAPTER 2

STRESS RELAXATION BEHAVIOR OF GLUTEN FOR US WHEAT CULTIVARS

2.1 Materials and methods

2.1.1 Materials

Two sets of wheat cultivars were investigated. One set of fifteen wheat cultivars was obtained from certified seed representing five US wheat classes, harvested in 2005: Hard Red Winter (HRW), Hard Red Spring (HRS), Soft Red Winter (SRW), Hard White (HDWH) and Soft White (SWH). The second set of 21 wheat cultivars are all HRW and include some experimental wheat cultivars. They were milled into flours using a Buhler Mill model MLU-202 following approved method 26-21A (AACCI 2000). These cultivars were chosen because they represent a good variety of wheat's commonly grown in the Midwest of the USA, and have been partially characterized in terms of their protein content, high molecular weight glutenin subunit (HMW-GS) composition, glutenin macropolymer (GMP) content, zeleny sedimentation volume (ZSV), and bread loaf volume (BLV), among others.

2.1.2 Sample preparation

All gluten samples were prepared for testing with a Glutomatic 2202 gluten washer (Perten Instruments AB, Huddinge, Sweden), as described by Zhao, et. al. (2010), with the exception that only single 10 g flour samples were washed at one time, which were not centrifuged subsequently. In addition a new press made out of plastic with a 2.5 mm gap was used instead of the steel plates, allowing for sequential pressing, and no Velcro dots were used to adhere the gluten to the TA-XT_{plus}. After relaxing the sample for 60 minutes a gluten sample was cut out with a sharp cookie cutter in a dog bone shape measuring 17.5 mm * 25.1 mm, with the central section

tapered to 12.7 mm * 10 mm. As described by Zhao et al (2010) a windowpane technique out of paper board of ~ 30 mm * 30 mm was used to allow the gluten samples to be clamped to the TA-XT_{plus}. The paperboard windowpane has a cutout in the center measuring 12.7 mm * 20 mm so as to hold the gluten sample at each end only. Attaching the gluten directly to a small piece of card-stock cutouts, and using double sided tape to attach the card-stock to the instrument was deemed an improvement without affecting the measurements.

2.1.3 Large deformation tensile and stress relaxation test

A texture analyzer (TA-XT_{plus}, Texture Technologies, Scarsdale, NY) with a 5 kg load cell and tensile grips was used. The rate of extension was 1 mm/s to a total strain (L/L_o) of 300-800% based on the crosshead movement. The samples were held at maximum strain for another 600 seconds, allowing the sample to relax to its equilibrium force (F_{equi}). Equilibrium was defined as the force reached after 600 seconds, from the beginning of the tensile test as this was deemed to be a reasonable time scale before significant sample drying occurred. All tensile tests were done at least in triplicate, each starting from a 10 g flour sample. All tensile test data was collected at room temperature. Since it was not possible at the time to raise the humidity level around the tested gluten samples, some degree of water loss was apparent after the 10 minute testing period, nonetheless this would be consistent for all samples, allowing for cross comparison.

2.1.4 Zeleny sedimentation test

Approved method 56-61A (AACCI 2000) was used to determine the sedimentation value for each cultivar. Values represent averages of duplicates.

2.1.5 Protein content and composition

During protein fraction extraction, all protein precipitation steps with acetone (40 and 80%) at -20°C were extended from 24 to 48 hr. The total protein in each

fraction was determined using approved method 46-30 (AACCI 2000) based on Dumas's nitrogen combustion in a LECO FP-528 nitrogen analyzer (LECO Corporation, St Joseph, MI). EDTA was used as standard and the protein to N ratio was 5.7.

2.1.6 HMW-GS/LMW-GS

The allelic variations of HMW-GS were determined in one dimensional sodium dodecyl polyacrylamide gels according to the method described by (Pflüger et al., 2001) with the following modifications: gliadins were not extracted and a resolving gel of 12% acrylamide was used. HMW-GS alleles were identified using standard cultivars and the method of (Payne & Lawrence, 1983) and (Shan et al., 2007).

2.1.7 Glutenin macropolymer (GMP)

GMP extraction was based on the methods reported by Graveland et al (Graveland et al., 1982) and Don et al (Don et al., 2005), where the protein content of the flour and GMP extractions were determined by the Dumas combustion method (AOCS 2004). Analysis was done in duplicate.

2.1.8 Bread loaf volume

Pup loaves were baked using approved method 10-10B (AACCI 2000). Fermentation time was 180 min; proof time 55 min and loaves were baked at 425 ˚F for 15 minutes. Mixing times varied from short (< 4 min) to normal (4.5 – 6 min) to slightly long (> 6.5 min). Absorptions were normal (62.5 – 65 %) except for McNeal, which had an absorption of 67%.

2.1.9 Mixograph

Mixograms were run on a 35-g Mixograph at their optimum water absorption (National Manufacturing Div., TMCO, Inc., Lincoln, NE) using approved method 54-40A (AACC International., 2009).

2.1.10 Statistical analysis

All cultivars were tested in at least triplicate, unless otherwise noted, using independently prepared samples. All errors are standard errors. Simple bivariate Pearson correlations were calculated for all variables. Statistical software SPSS® Release 18 (SPSS, Chicago, IL, USA) was used for the data analysis.

2.2 Results and discussion

As shown previously by Zhao, et. al. (2010), the tensile strength of wheat gluten from different cultivars can be clearly differentiated using a large deformation uni-axial tensile test. Fig. 7 shows the large deformation stress-relaxation curves for the selected 15 wheat cultivars extended to 5x their original length, as a function of force over time. Fig. 10 shows the same information for the 21 HRW wheat cultivars. With the current experimental setup it was not possible to accurately measure the sample cross section upon extension; therefore Fig. 7 and 10 show the change of force with strain, rather than true stress vs. strain. Considering there is no strong reason to suggest different sample cross sections near equilibrium, it seems acceptable to compare the measured force parameters of those cultivars instead of true strain. Nonetheless it is possible to approximate the sample cross section if one assumes its volume remains constant during stretching. A true stress-strain curve of the tested gluten samples would show strain hardening.

During the relaxation period, the gluten approaches an equilibrium force. Nonetheless it is not possible to reach a true equilibrium due to the vertical setup, as gravity continues to act on the sample throughout the experiment. The 15 cultivars

show a wide spread of maximum forces at 500% extension, ranging from 0.042 N for Stephens to a high of 0.431 N for McNeal, with each cultivar having a clearly defined extension and relaxation pathway (Fig. 7, Table 3). Equally, the 21 HRW wheat cultivars show a 4 fold spread in the maximum force reached at 500% extension (Fig. 10, Table 4), despite their similar protein chemistry.

Interestingly the extension and relaxation pattern for each cultivar was similar, and when each curve is normalized as shown in Fig. 8 and 11, they superimpose. This superposition effect indicates also that the relaxation times for the gluten are similar, suggesting that the internal dynamics of stress relaxation are comparable. Nonetheless previous small deformation stress-relaxation studies over equally long relaxation time periods with dough, purified gluten and gluten protein fractions show two distinct relaxation phenomena (Li et al., 2003), and also distinct relaxation times for semolina dough ranging from strong to weak durum wheat cultivars (Rao et al., 2001). This is not too surprising considering most small deformation oscillatory studies have shown limited applicability for wheat end use properties under common dough processing conditions (Dobraszczyk & Morgenstern, 2003). Furthermore shear strain has been shown to have different physical effects on HMW polymers than during uni-axial extension, especially for branched polymers (Ferry, 1980).

Considering the maximum force reading at full extension, the force dissipated over time would represent the rearrangement of the gluten polymer network, whereas the equilibrium force represents the true elastic component of the maximum force. As a result the degree of elasticity (DE), which is the ratio of un-dissipated to total force at equilibrium, of the representative cultivars is also similar, ranging from 31-43% at 500% extension, outliers being Stephens and Roane (Table 3). Both Stephens and Roane have 2 + 12 HMW subunits, although TAM 110 does as well and has a relatively high DE, indicating that any differences in DE cannot be explained solely by

HMW subunits or wheat class. The DE for the 21 HRW cultivars ranges from 36-46% (Table 4). These results mirror the data presented by Zhao, et. al. (2010) who calculated the degree of elasticity by comparing the work of extension to the work of retraction for their large deformation tensile test. As such the degree of elasticity parameter is not suitable to differentiate clearly between wheat glutens, compared to the gluten strength data.

Zhao, et. al. (2010) showed that under small deformation creep recovery conditions, these particular glutens exhibit almost complete stress relaxation at small time scales, and a high degree of recoverability at long creep, and creep recovery times. This contrasts with this large deformation stress-relaxation experiment, which shows gluten relaxing only approximately 60% of the initially applied stress. This suggests that these large strains cause irreversible changes in the gluten matrix.

To determine the resistance of gluten to different strains, the degree of elasticity was measured at different extensions, ranging from 300% - 700% for 9 of the 15 cultivars. As shown in Figure 9, the force at equilibrium increases linearly with the crosshead displacement, indicating that the elastic component of the glutens follows Hooke's Law across greater than two fold changes in length under these time conditions, i.e. the extension of the spring is in direct proportion to a force (below the materials elastic limit) exerted on it ($F = -kx$, where F = Force; k = spring constant; x = linear displacement). The slope of the curves then corresponds to the spring constant. These range almost 8x from Stephens at 0.0017 N/cm to Briggs at 0.0187 N/cm (Appendix A). The spring constant of the elastic component of gluten could be used as an alternative indicator of gluten strength, and can be used to calculate the elastic force at different strains, although it would be worthwhile to investigate whether the spring constant is independent of strain rate.

The tensile strength of polymers was first modeled by an equation introduced by Flory, which related tensile strength to a threshold molecular weight and the number average molecular weight of the polymers. This equation was further refined to account for polydispersed polymers by adding a term to the equation accounting for the fraction of polymers with a MW greater than the threshold MW:

$$\sigma = \sigma_o(1-M_T/M_n)\,\Theta \qquad (1)$$

where σ is the tensile strength; σ_o = the limiting tensile strength at high MW; M_T = a threshold MW; Θ is the fraction of polymers with $M > M_T$; and M_n is the number average molecular weight of this fraction (Singh & MacRitchie, 2001). As a result dough and gluten strength has been previously investigated in terms of protein content, molecular weight distribution, subunit types, amongst others (Gupta et al., 1993). Table 5 lists the Pearson correlation coefficients for simple bivariate comparisons of gluten strength, i.e. F_{max} at 500% extension for the set of 15 wheat cultivars vs. a variety of known physicochemical and rheological parameters. On the whole gluten strength correlates well with other gluten tests, including the DR measured by the CORE analyzer (see Chapter 3), gluten index, the spring constant, and the Glutograph peak and final values. As reported by Zhao, et. al. (2010) gluten strength also correlates with mixograph mixing time, which is surprising since dough is a very heterogenous system with much more variable gluten content. Nonetheless it makes sense that it would require a greater energy input to properly develop and disperse a tougher gluten network.

Previous studies have argued that tensile strength is determined mostly by the abundance of insoluble, large molecular weight proteins in gluten, rather than overall protein content. Therefore the quantity of glutenin macropolymer in flour should be

predictive of gluten and dough strength. Nonetheless there exist conflicting results on the effect of polymeric protein and GMP content on dough strength measured by the extensigraph and mixograph (Gupta et al., 1993), which is also reflected by the poor correlation of 0.409 between gluten strength of the 15 cultivars and their GMP quantity obtained in this study (Table 3). Overall GMP yield %, which is a measure of the amount of GMP protein extracted to the overall flour protein content, gives an even poorer correlation of 0.074 with gluten strength (data not shown). A possible explanation is that the GMP protocol requires the gluten to be denatured and solubilized in a sodium dodecyl sulfate solution, thereby negating many structural and less permanent entanglement effects that influence both MW distribution and tensile strength. In addition there are many environmental effects such as annual precipitation levels and the application of fertilizers that affect gluten strength of wheat, which this study does not control for.

There exists a fairly good understanding of which genetic factors can contribute to good quality wheat flours, based mostly in terms of BLV criterion. Once a certain "quality" of gluten has been reached, overall protein content becomes a good predictor of BLV. This is corroborated by the data shown in Table 1, since the correlation between BLV and protein content on a 14% MB has a Pearson correlation of 0.778, and the correlation between BLV and ZSV an even better Pearson correlation of 0.862. Dobraszczyk et. al. did a principle component analysis of various rheological and physical gluten and dough parameters, and created a more complete model for predicting BLV (Dobraszczyk & Salmanowicz, 2008).

The good correlation between gluten strength and degree of recovery measured with the CORE analyzer makes sense, since gluten strength is directly related to the degree of elasticity measured by the texture analysis, i.e. more elastic gluten would have a greater snap back, or degree of recovery during a bi-axial compression and relaxation test. The CORE analyzer will be described in greater detail in the following chapter.

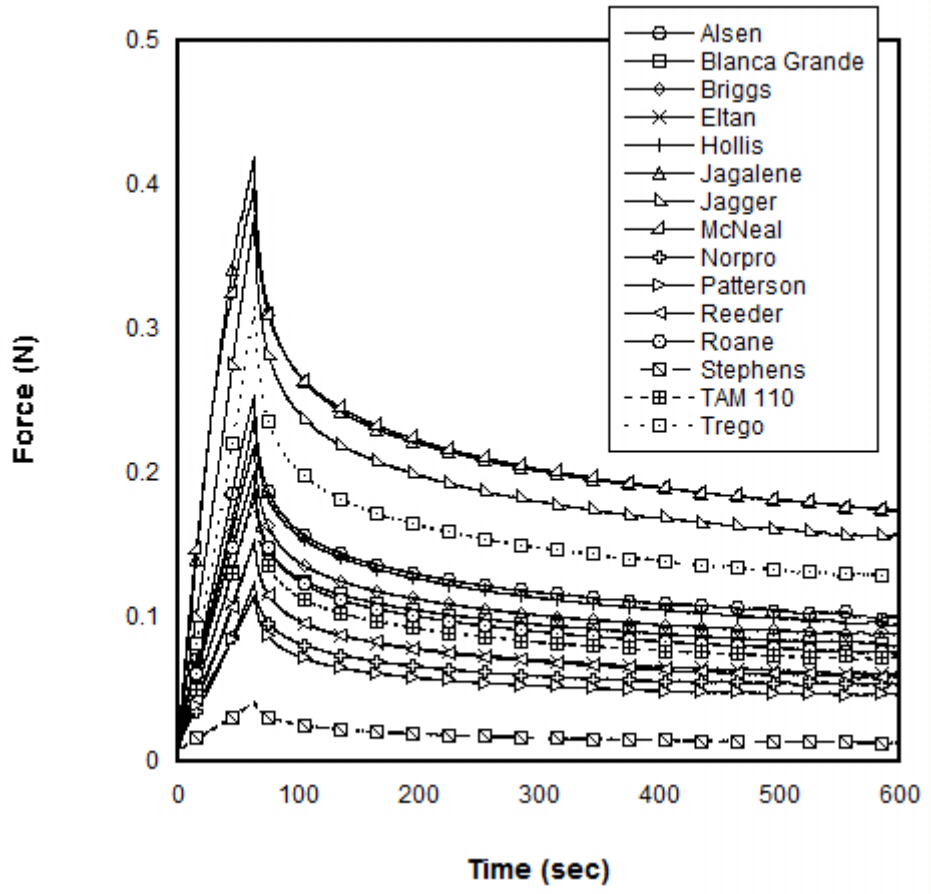

Figure 7 Uni-axial stress relaxation behavior of gluten obtained from different wheat cultivars.

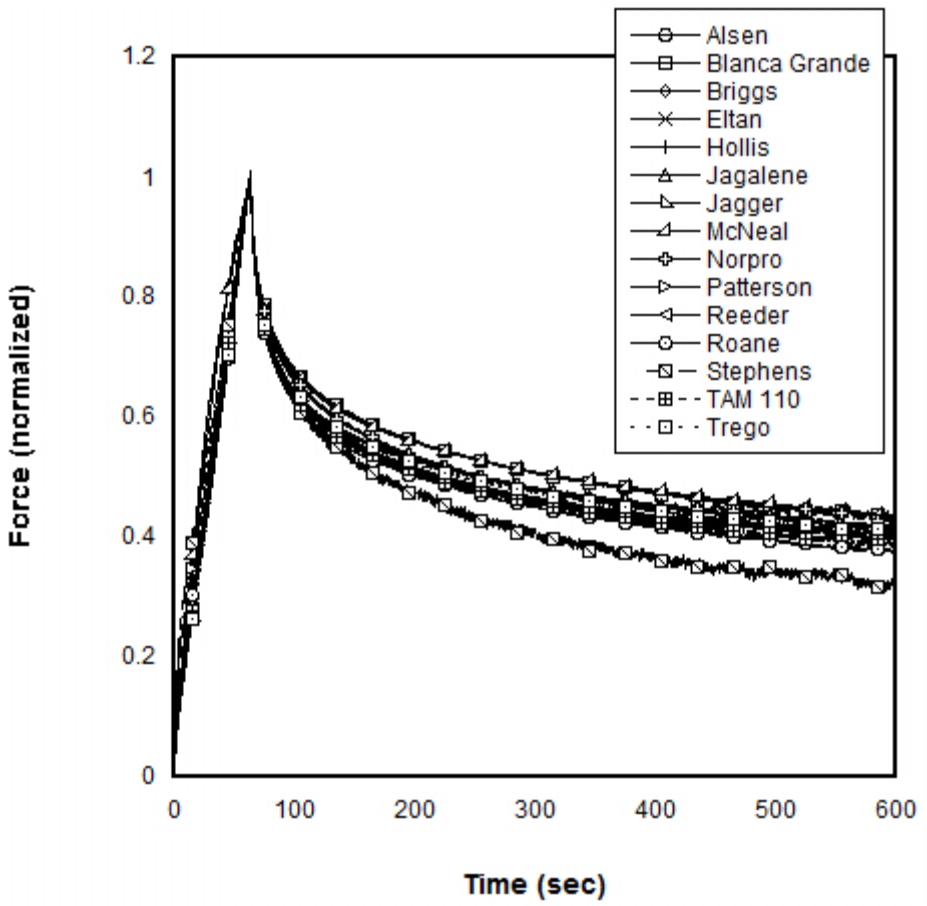

Figure 8 Superposed stress relaxation behavior of gluten obtained from different wheat cultivars.

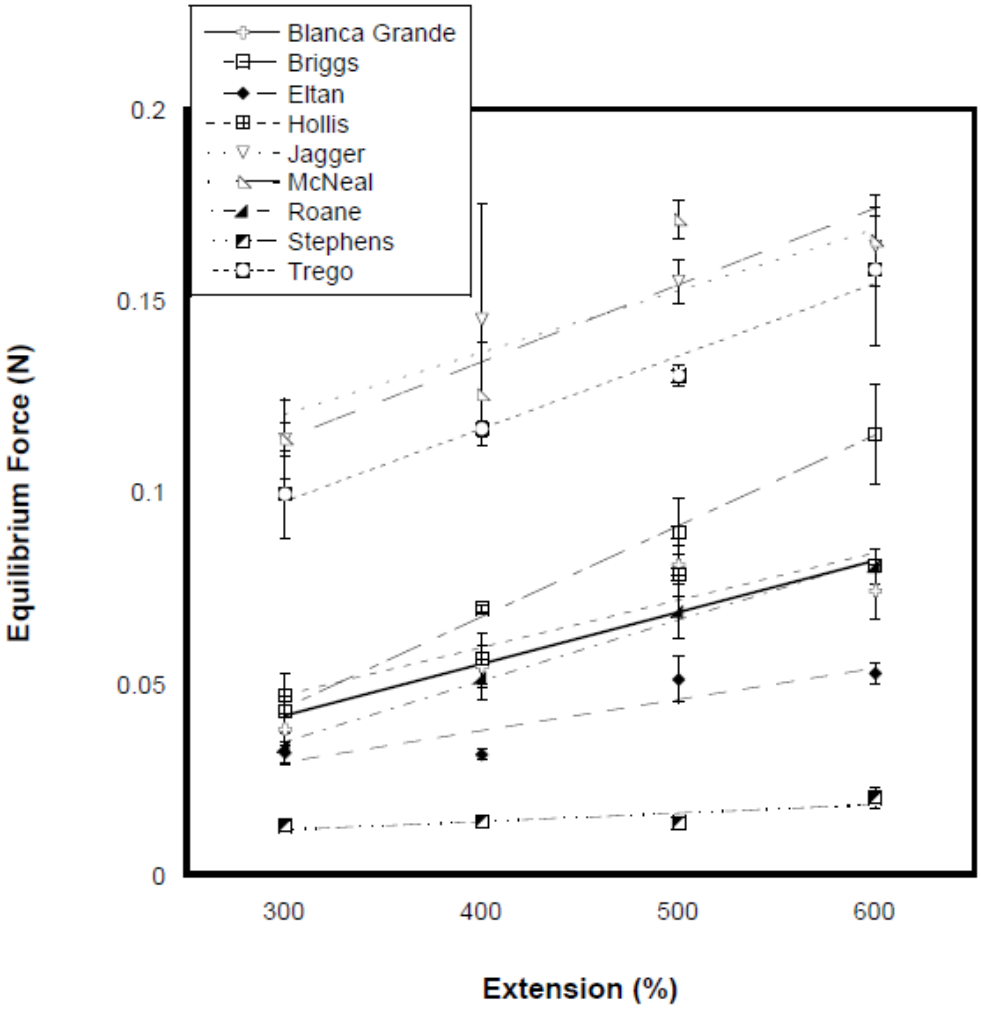

Figure 9 Equilibrium forces determined at different extensions for select cultivars vs. strain.

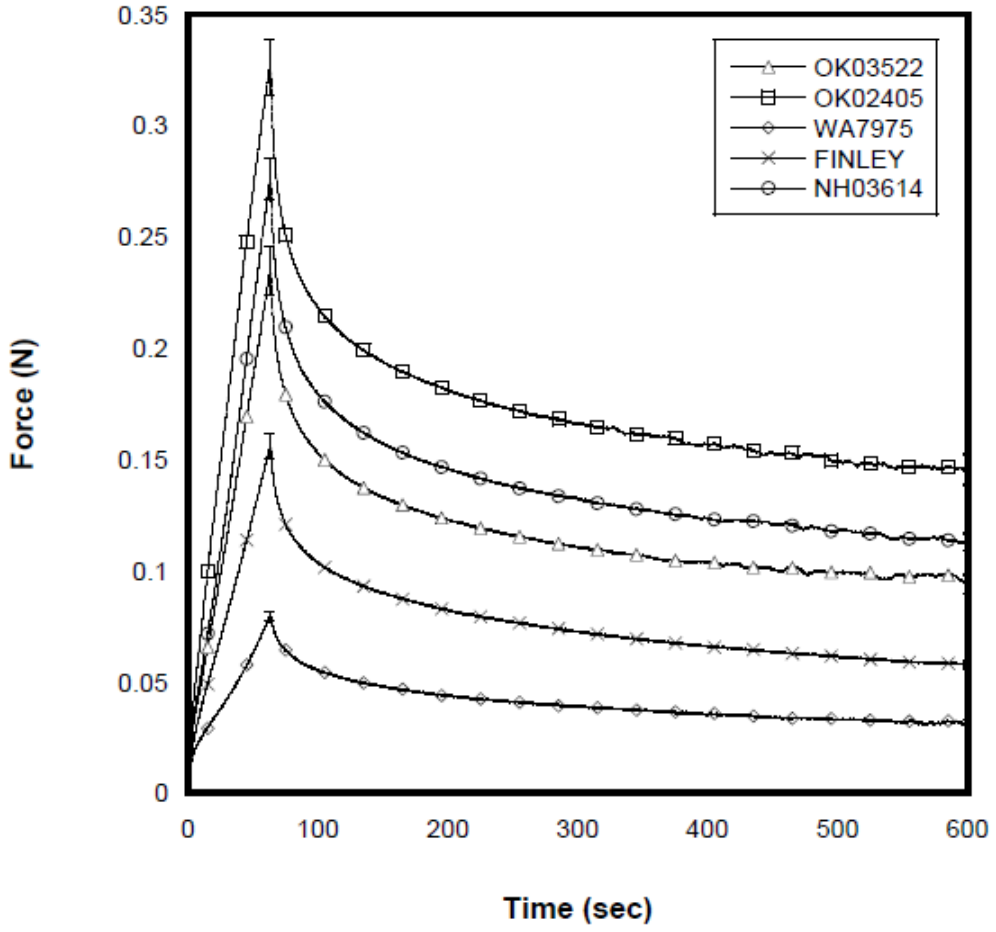

Figure 10 Uni-axial stress relaxation behavior of gluten obtained from different HRW wheat cultivars.

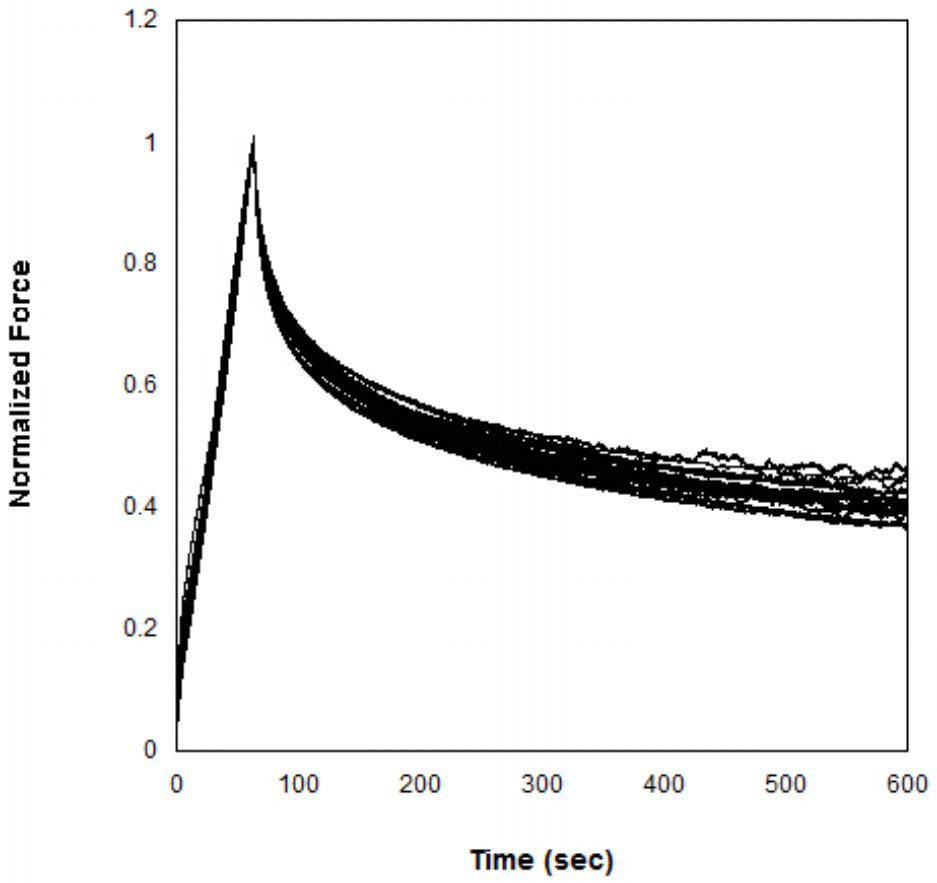

Figure 11 Superposed stress relaxation behavior of gluten obtained from different HRW wheat cultivars.

Table 1

Phyiscochemical properties of 15 US wheat cultivars representing HRS, HRW, HDWH, SRW, SWH wheat classes

	HMW-GS Alleles				Glutenin Macropolymer			
Cultivar	1A	1B	1D	Protein Content (%)*	Yield (%)	Weight (g)	Protein Quantity (mg)	BLV (mL)
HRW								
Tam 110	2*	7+8	2+12	14.04±0.05	9.12	1.44	18.43	918.75 ± 9.38
Jagger	1	17+18	5+10	11.21±0.02	12.22	1.51	20.69	832.25 ± 5.98
Jagalene	1/2*	17+18	5+10	9.98±0.07	9.92	1.18	11.68	768.75 ± 9.38
HRS								
Alsen	2*	7+9	5+10	15.96±0.07	9.71	1.92	29.76	918.75 ± 12.88
Briggs	1/2*	7+9	5+10	13.52±0.06	12.06	1.63	26.57	825.00 ± 5.10
McNeal	1	17+18	5+10	14.28±0.05	11.34	1.93	31.27	956.25 ± 7.86
Reeder	2*	7+9	5+10	13.11±0.04	8.31	1.39	15.15	856.25 ± 5.98
Hollis	2*	17+18	5+10	13.01±0.04	12.22	1.60	25.44	881.25 ± 23.59
Norpro	2*	7+9	5+10	12.04±0.1	8.64	1.36	14.14	787.50 ± 6.25
HDWH								
Blanca Grande	1	17+18	5+10	13.15±0.08	11.63	1.98	30.29	912.50 ± 3.61
Trego	2*	14+15	5+10	10.34±0.04	19.05	1.32	26.00	743.75 ± 20.01
SRW								
Patterson	1	7	5+10	8.49±0.09	20.26	0.94	16.17	737.50 ± 14.88
Roane	null	7+8	2+12	7.69±0.02	16.64	1.14	14.59	687.50 ± 8.07
SWH								
Stephens	null	7+9	2+12	11.62±0.03	8.18	1.01	9.60	675.00 ± 7.22
Eltan	1	7	5+10	10.93±0.04	13.08	1.35	19.31	862.50 ± 11.97

* 14% Moisture basis

Table 2

Physicochemical properties of 21 US HRW wheat cultivars

Cultivar	Protein Content (%)*
1-UT9743-42	11.17
GARLAND	11.81
IDO653	13.12
IDO651	13.01
UI DARWIN	11.95
WA7975	10.85
FINLEY	10.74
IDO621	12.5
BOUNDARY	11.62
UT9325-55	11.84
PROMONTORY	11.69
Millenium	12.34
NH03614	11.355
OK Bullet	12.79
OK00514-05806	11.955
OK03522	11.11
OK02405	12.4
SD01058	12.185
SD0111-9	12.72
SD01273	11.375
MT0495	11.175

* 14% Moisture basis

Table 3

F_{max}, F_{equi}, DE (ratio of F_{equi} and F_{max} * 100%) and DR (ratio of distance recovered and distance compressed *100%) for the set of 15 wheat cultivars

Cultivar	F_{max} (N)†	F_{equi} (N)††	Degree of Elasticity (%)	Degree of Recovery (%)
HRW				
Tam 110	0.207 ± 0.025	0.081 ± 0.009	39.13 ± 6.31	47.03 ± 2.86
Jagger	0.383 ± 0.009	0.155 ± 0.006	40.47 ± 1.75	74.80 ± 1.50
Jagalene	0.419 ± 0.025	0.174 ± 0.013	41.53 ± 3.41	77.65 ± 0.79
HRS				
Alsen	0.253 ± 0.015	0.099 ± 0.010	39.13 ± 5.61	68.94 ± 0.84
Briggs	0.219 ± 0.019	0.089 ± 0.008	40.64 ± 5.37	57.96 ± 3.33
McNeal	0.431 ± 0.024	0.177 ± 0.007	41.07 ± 3.12	-
Reeder	0.165 ± 0.015	0.065 ± 0.007	39.39 ± 5.43	50.58 ± 7.45
Hollis	0.235 ± 0.015	0.090 ± 0.008	38.30 ± 2.42	68.75 ± 1.24
Norpro	0.124 ± 0.011	0.054 ± 0.004	43.55 ± 4.82	41.88 ± 5.07
HDWH				
Blanca Grande	0.188 ± 0.015	0.074 ± 0.005	39.36 ± 3.64	62.77 ± 1.74
Trego	0.314 ± 0.009	0.130 ± 0.003	41.40 ± 1.45	69.91 ± 3.91
SRW				
Patterson	0.115 ± 0.013	0.046 ± 0.004	40.00 ± 5.64	-
Roane	0.194 ± 0.009	0.069 ± 0.007	35.57 ± 4.04	31.83 ± 2.78
SWH				
Stephens	0.042 ± 0.004	0.013 ± 0.001	30.95 ± 3.27	5.02 ± 0.35
Eltan	0.130 ± 0.015	0.051 ± 0.006	39.23 ± 6.37	29.12 ± 3.14

† at 500% extension

†† at 500% extension after 600 seconds

Table 4

F_{max},F_{equi} and DE (ratio of F_{equi} and F_{max} * 100%) for the set of 21 wheat cultivars

Cultivar	F_{max} (N)†	F_{equi} (N)††	Degree of Elasticity (%)
1-UT9743-42	0.270 ± 0.024	0.115 ± 0.010	42.59
GARLAND	0.080 ± 0.006	0.031 ± 0.002	38.75
IDO653	0.089 ± 0.004	0.034 ± 0.003	38.20
IDO651	0.142 ± 0.006	0.052 ± 0.002	36.62
UI DARWIN	0.111 ± 0.007	0.046 ± 0.004	41.44
WA7975	0.079 ± 0.005	0.032 ± 0.003	40.51
FINLEY	0.156 ± 0.011	0.058 ± 0.004	37.18
IDO621	0.093 ± 0.005	0.037 ± 0.001	39.78
BOUNDARY	0.124 ± 0.006	0.053 ± 0.002	42.74
UT9325-55	0.236 ±0.015	0.088 ± 0.005	37.29
PROMONTORY	0.231 ± 0.025	0.099 ± 0.008	42.86
Millenium	0.132 ± 0.010	0.053 ± 0.007	40.15
NH03614	0.276 ± 0.021	0.113 ± 0.009	40.94
OK Bullet	0.136 ± 0.012	0.054 ± 0.003	39.71
OK00514-05806	0.190 ± 0.022	0.077 ± 0.007	40.53
OK03522	0.235 ± 0.025	0.094 ± 0.009	40.00
OK02405	0.326 ± 0.025	0.145 ± 0.014	44.48
SD01058	0.164 ± 0.020	0.073 ± 0.006	44.51
SD0111-9	0.105 ± 0.009	0.048 ± 0.003	45.71
SD01273	0.158 ± 0.014	0.065 ± 0.006	41.14
MT0495	0.185 ± 0.011	0.078 ± 0.001	42.16

† at 500% extension

†† at 500% extension after 600 seconds

Table 5
Correlations between gluten strength of the 15 wheat cultivars and their relevant physicochemical properties

Parameter	Pearson Correlation	
Protein 14% MB	0.134	
Zeleny sedimentation volume	0.408	
GMP protein quantity	0.409	
Pup loaf volume	0.337	
Mixograph mix time	0.879	**
CORE %DR	0.855	**
Gluten Index	0.712	**
Gluten spring constant	0.721	**
Glutograph-E peak	-0.844	**
Glutograph-E final	-0.795	**
Alveograph P	0.593	
Alveograph W	0.554	
Alveopgraph L	0.242	
Farinograph development time	0.349	
Farinograph stability	0.577	

**. Correlation is significant at the 0.01 level (2-tailed).

2.3 Conclusions

Gluten tensile testing is effective at further differentiating wheat cultivars of different genetic and physicochemical makeup, but similar wheat classes. These gluten tensile tests have shown that gluten strength is strongly correlated with the elastic component of wheat gluten, which is a good predictor of the optimum mixing time of dough. As a result flour strength can be measured and adjusted to accommodate more desirable dough mixing and handling behavior.

In addition these experiments have shown a highly linear correlation between gluten strength, gluten elasticity and strain, allowing for a spring constant approximation for specific gluten, from which its behavior at different strains can be predicted. The spring constant could be another useful parameter for predicting the elastic component of similar gluten samples at different strains. Nonetheless gluten strength is not a good predictor of bread loaf volume, which remains most closely correlated with overall protein content and zeleny sedimentation value.

Furthermore the data presented does not support the hypothesis that large molecular weight subunits, as measured by GMP quantity and GMP % of total flour protein explain gluten strength. At this junction it is unclear which factors underlie the great variation in gluten strength exhibited by the tested wheat cultivars, although polymer science suggests that it would most likely be closely related to the quantity of cross links and possible entanglements.

Future efforts to understand the factors relating to gluten strength and elasticity should focus on molecular level structure-function effects. Many exciting opportunities exist to apply a variety of different molecular tools to study gluten structure and function, including sequencing, genetic modification, and different imaging techniques. Optical tweezers would also lend themselves well to studying rheology at the molecular level, rather than studying complex systems as a whole on a

macroscopic level. Perhaps small deformation oscillatory rheology could also be combined with large deformation rheology to get the best of both worlds.

CHAPTER 3

CORE ANALYSIS OF GLUTEN EXTRACTED FROM COMMON US WHEAT CULTIVARS

3.1 Introduction

Many rheological tools exist, ranging from mostly empirical to more fundamental measurements. All the more there are many designed especially for assessing food properties, such as a sauces viscosity, the texture and firmness of baked bread, or the extensibility of dough, for instance. Within the gradation of empirical and fundamental tests there also exists the delineation of large- and small-deformation dynamic tests. Many of the rheological techniques relating to bread making are reviewed by Dobraszczyk (Dobraszczyk & Morgenstern, 2003).

Within this large realm of rheological tools that can be adapted to study many different food systems, Perten Instruments has developed a rapid, fundamental bi-axial extension instrument specifically made to test wheat gluten elastic recovery (CORE analyzer - See Fig. 13), but can also be easily adapted for use with dough and other visco-elastic materials. The main advantages of this testing system are the instruments sensitive load cell, its small form factor, and the speed at which a test can be conducted, at least in comparison with the TA-XT_{plus} experiment described above, which includes an hour resting period for each sample. Beyond this the advantage of a bi-axial compression system are that the sample only has to be placed into the testing device, rather than attached and stretched. Furthermore bi-axial extension is more closely related to the strain gluten undergoes during gas bubble expansion.

Even for wheat cultivars with similar genetic makeup and protein chemistry (see Table 1 and 2), there exists great variation in overall gluten strength, defined as a force measurement taken at a certain extension of a gluten sample (See Fig. 7 and 10).

Since neither overall protein quantity, the cultivars genetic makeup, wheat class, or other chemical analyses give a good understanding or prediction of gluten strength and its elastic recovery properties, a quicker, more accurate and reproducible instrument was developed to improve on the TA-XT_{plus} system described in the previous chapter.

3.2 Materials and methods

3.2.1 Materials

The same flours were used as for the first experiment described in chapter 2, except that due to quantity constraints, cultivars McNeal and Patterson could not be extensively analyzed and therefore these data have been omitted.

3.2.2 CORE sample preparation

All gluten samples were prepared by washing 2 x 10g of flour in Perten Instruments Glutomatic 2202, after which the pellets were loaded into a cylindrical shaper with a closely fitted plunger (see Fig. 12). The pellets were centrifuged in the shaper for 5 minutes in Perten Instruments Centrifuge 2015 at 6000 rpm, creating a uniform cylindrical sample that could easily be loaded into the CORE analyzer. All CORE experiments were conducted in a temperature controlled room at 21 ºC.

3.2.3 CORE assay

The shaped gluten samples were allowed to rest for 1-2 minutes before loading into the pre-calibrated CORE analyzer (See Fig. 13). The gluten samples were then subsequently compressed for 5 seconds with a peak force of 8N, and allowed to recover over a further 55 second period. The CORE analyzer recorded the compression distance as a factor of time over a one minute interval. At least three replicates for each cultivar were obtained from independently washed flour samples. The CORE analyzer is designed to work with the Glutomatic gluten washer from Perten Instruments, and their centrifuge.

Figure 12 The individual components of the gluten shaping tool used in conjunction with the centrifuge provided by Perten Instruments, include the bottom bracket (top) which attaches to the rotor via a pin, and holds the plastic cylinder (bottom right) with a gluten sample held between the bottom bracket and the piston (bottom right).

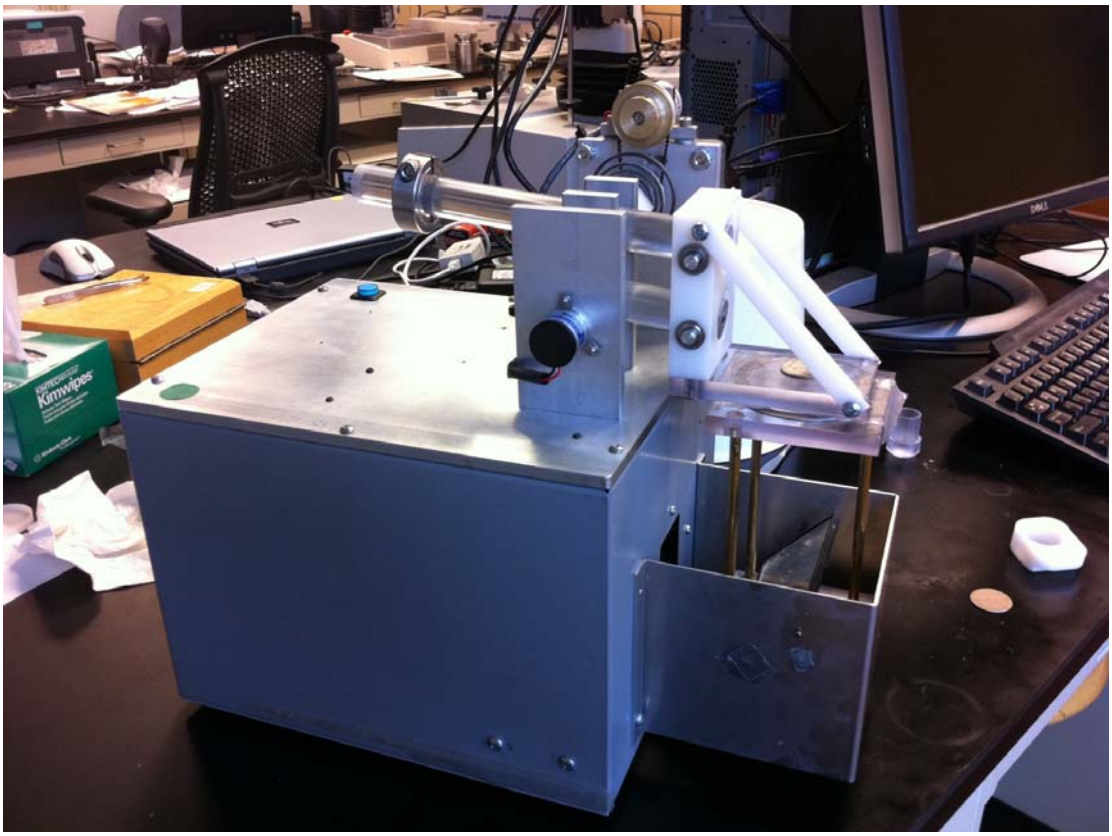

Figure 13 The prototype CORE analyzer.

3.2 Results and discussion

Figure 10 shows the normalized compression data for the gluten washed from the set of 15 wheat cultivars. After the 5 second hold period with a compression force of 8 N, each gluten sample shows some degree of elastic recovery. The degree of recovery, defined as the ratio of the overall distance recovered over the distance compressed is listed in Table 3 alongside their gluten strength measurements. The degree of the recovery ranges from a low of 5.07 % for the Stephens cultivar to 77.34 % for Jagalene, mirroring the behavior of gluten strength measured with the TA-XT_{plus} tensile test. Indeed, gluten strength correlates well with the degree of recovery measured with the CORE analyzer (see Fig. 15). This suggests that the behavior of the various gluten networks is comparable under both uni-axial and bi-axial extension conditions.

Nonetheless friction effects dominate for weaker glutens, providing a possible explanation for drop-off in the degree of recovery for the Stephens cultivar, and would indicated that a linear fit is not appropriate. A material with no elastic strength would not show any compression-recovery; therefore the fitted line can be forced through the origin. Presumably the degree of recovery will plateau at higher gluten strengths; nonetheless more, stronger wheat cultivars should be investigated to validate this. Perhaps rather than using the CORE analyzer for creep-recovery and measuring the degree of recovery, the instrument could be measure stress relaxation instead. This would allow easier comparison with more traditional tensile tests. Furthermore if the camera apparatus could be used to measure the sample cross sectional area, true stress-strain data could be measured.

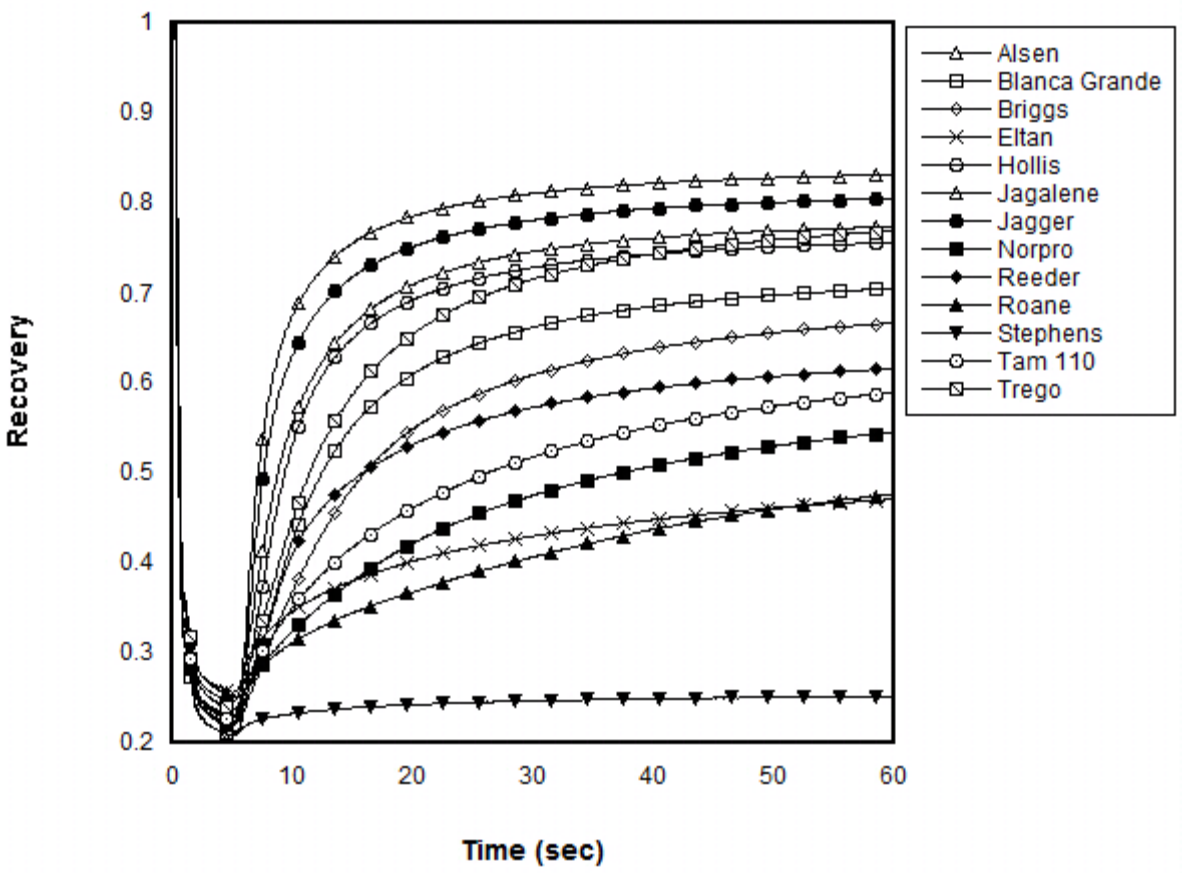

Figure 14 Recovery curves of gluten obtained from different wheat cultivars after compression in the CORE analyzer.

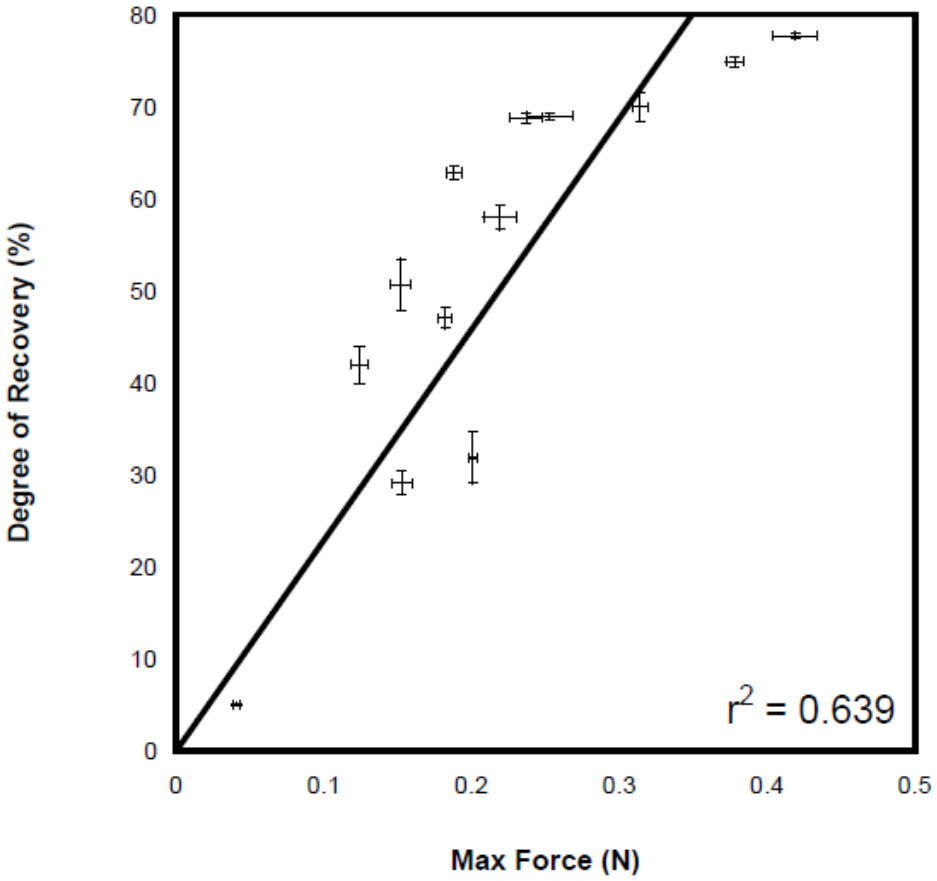

Figure 15 Correlation of DR and F_{max} at 500% extension.

3.3 Conclusions

The CORE analyzer is designed and suited well for a quick assessment of gluten strength and degree of recovery, which is helpful in predicting optimum mixing times and differentiating wheat cultivars in terms of gluten strength. Further advantages of the CORE analyzer over the tensile test described in Chapter 2 are that the gluten sample does not have to be rested, and can be placed directly into the apparatus, rather than suspended and stretched. In addition the CORE analyzer integrates well with the centrifuge and gluten washer made by Perten Instruments, and can be adapted to test other soft solids.

Future efforts should focus on more fully understanding the relationship between the degree of recovery and gluten strength. The CORE instrument would be a good competitor for current texture analyzer systems if it can be modified to be more versatile in terms of the tests it can run and the measurements it can make.

CHAPTER 4

STRESS RELAXATION BEHAVIOR OF DOUGH MADE FROM COMMON US WHEAT CULTIVARS

4.1 Introduction

Turning wheat flour into a dough and ultimately bread requires significant mechanical work input during kneading and sheeting. In addition dough undergoes significant physical changes during proofing and baking, therefore several empirical dough rheology instruments have been developed to better understand dough behavior during processing and the effects of other additional ingredients (Dobraszczyk & Morgenstern, 2003). These include the Alveograph, Brabender extensigraph and Kieffer dough and gluten extensibility rig.

The Alveograph measures the extensibility and resistance to stretching by inflating a sheet of dough into a bubble until it ruptures. The Brabender extensigraph and Kieffer dough and gluten extensibility rig are based on similar principles, but differ mostly in the sample sizes they use. By pulling on a strip of dough clamped at both ends with a hook, these two instruments measure the extensibility and resistance to extension of dough, and can give an indication of the amount of work needed for a certain degree of extension. The benefit of the Kieffer extensibility rig are that it can be performed on a 0.4 g sample, compared to 300 g of dough for the Brabender extensigraph, and can be adapted to most materials testing machines. This allows for more fundamental measurements of stress and strain (Sliwiniski, 2003). Nonetheless since a hook is used to pull a strip of dough, a hard to account for shear effect is introduced into the measurement.

As a proof of principle, the stress relaxation assay described in Chapter 2 was adopted for use with dough, building on the uni-axial extension tests described above,

but without the shear thinning problem introduced by the use of a hook. This experimental set up also allows for measuring more fundamental rheological parameters, and an easy comparison with information gleaned from the previous gluten experiments.

4.2 Materials and methods

4.2.1 Materials

A selection of 8 flours from the 15 cultivars described in chapter 2 representing all 4 US wheat classes was used, these included Blanca Grande, Briggs, Eltan, Hollis, Jagger, Roane, Stephens, and Trego.

4.2.2 Sample preparation

According to AACC method 54-40A (AACCI 2000) flours were mixed to peak torque with the appropriate quantity of 2% salt water based on their absorbance determined by AACC method 54-40A. After the required mixing time, 10 g dough samples were pressed between two lubricated plates with a 2.5 mm gap, and allowed to rest for 1 hr before being shaped and loaded in the same way as described for gluten in chapter 2. In addition a new press made out of plastic with a 2.5 mm gap was used instead of the steel plates, allowing for sequential pressing, and no Velcro dots were used to adhere the dough to the TA-XT_{plus}. After relaxing the sample for 60 minutes a dough sample was cut out with a sharp cookie cutter in a dog bone shape measuring 17.5 mm * 25.1 mm, with the central section tapered to 12.7 mm * 10 mm. As described by Zhao et al (2010) a windowpane technique out of paper board of ~ 30 mm * 30 mm was used to allow the gluten samples to be attached to the TA-XT_{plus}. The paperboard windowpane has a cutout in the center measuring 12.7 mm * 20 mm so as to hold the gluten sample at each end only. Attaching the dough directly to a small piece of card-stock cutouts, and using double sided tape to attach the card-stock to the instrument was deemed an improvement without affecting the measurements.

4.2.3 Stress relaxation

A texture analyzer (TA-XT2^{plus}, Texture Technologies, Scarsdale, NY) with a 5 kg load cell and tensile grips was used. With an extension rate of 1 mm/s an L/L_o of 300% was reached for the fully relaxed and shaped dough samples. A relatively small strain was used because the limited extensibility of dough, which tended to rupture beyond an L/L_o of 300%. The samples were held at maximum strain for another 600 seconds, allowing the sample to reach equilibrium. All tensile tests were done at least in triplicate, starting from the same dough sample. All tensile test data was collected at room temperature. Since it was not possible at the time to raise the humidity level around the tested dough samples, some degree of water loss was apparent after the 10 minute testing period, nonetheless this would be consistent for all samples, allowing for cross comparison.

4.3 Results and discussion

As seen in Fig. 16 the stress relaxation behavior and overall tensile strength varies greatly across cultivars, although the overall behavior is reminiscent of the gluten data shown previously in Fig. 2 and 5. Equally, as shown in Fig. 17, if one normalizes for the maximum force obtained at equal extension, then the stress-relaxation curves superimpose nicely, indicating that the relaxation behavior up until about 200 seconds is comparable. Nonetheless considering the initial stress-strain behavior of dough (Fig. 18), the dough samples seem to have a two-phase behavior, with the exception of the Hollis cultivar, indicated by an initial greater resistance to extension, i.e. a more elastic response, followed by very gradual linear increase in stress, which might imply viscous flow after the elastic gluten network has been disrupted.

Since significant water loss occurred after 200 seconds and caused the dough to dry up and contract, the degree of elasticity was calculated as the ratio of the

equilibrium force at 200 seconds divided by the maximum force at 300% extension (See Table 6). The degree of elasticity for the dough from the respective wheat cultivars ranges from a low of 42 % for Hollis to a high of 49% for Jagger. This mirrors the behavior shown by gluten tested in chapter 2 under similar conditions from the same flours. Nonetheless gluten strength, as defined by the maximum force of the stress-relaxation curve for gluten, is not necessarily a good indication of dough strength, as shown by Fig. 19, even though gluten is believed to be the main contributor to dough elasticity and strength. Due to limited resources and time, only 8 flours were investigated, therefore it is difficult to make any assumptions about which clusters are truly outliers, although perhaps with a greater data set different groups might be identified. Adjusting for their relative protein content of the flours did not significantly improve the correlation between dough and gluten strength.

Table 6

F_{max}, F_{equi}, and DE (ratio of F_{equi} to F_{max}) for 8 doughs

Cultivar	Max Force (N)†	Equilibrium Force (N)	Degree of Elasticity %
Blanca Grande	0.101 ± 0.012	0.046 ± 0.005	45.545
Briggs	0.084 ± 0.009	0.039 ± 0.004	46.429
Eltan	0.031 ± 0.004	0.014 ± 0.002	45.161
Hollis	0.121 ± 0.021	0.051 ± 0.007	42.149
Jagger	0.067 ± 0.010	0.033 ± 0.005	49.254
Roane	0.033 ± 0.001	0.016 ± 0.001	48.485
Stephens	0.016 ± 0.002	0.007 ± 0.001	43.750
Trego	0.064 ± 0.003	0.031 ± 0.002	48.438

† at 300% extension

†† at 300% extension after 200 seconds

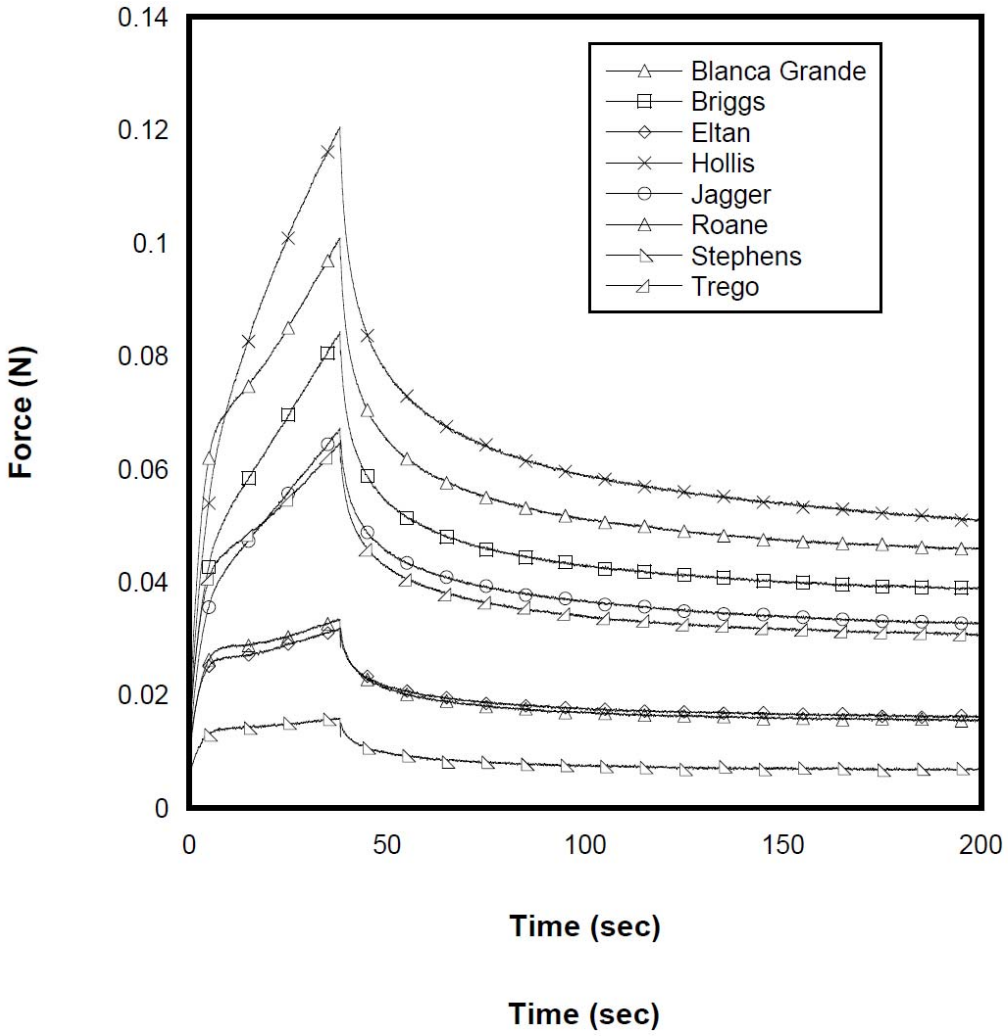

Figure 16 Stress – relaxation behavior of eight dough samples from representative wheat cultivars.

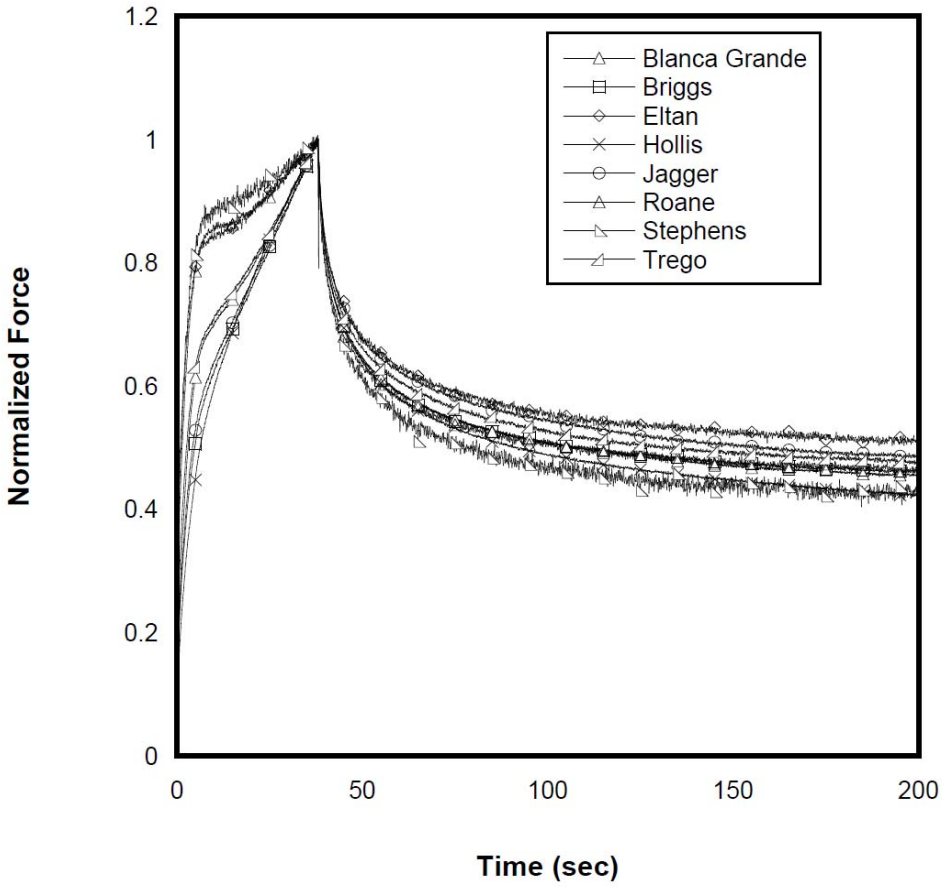

Figure 17 Superposed stress – relaxation behavior of eight dough samples from representative wheat cultivars.

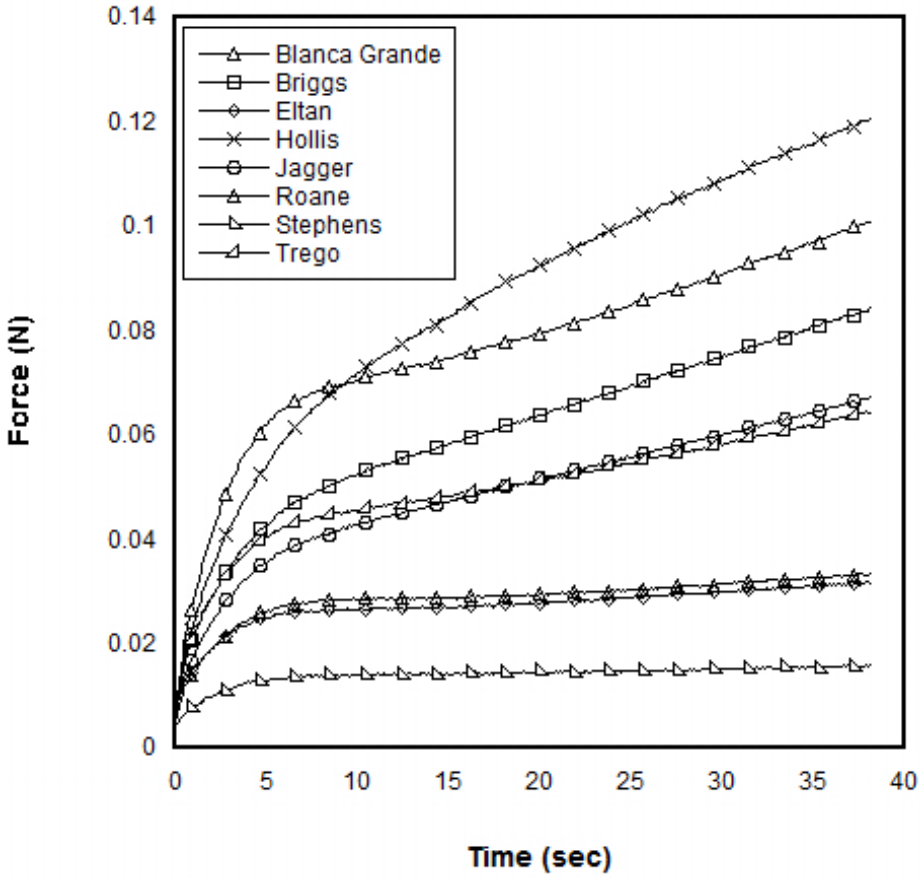

Figure 18 Initial stress-strain behaviors for dough during extension to 300%.

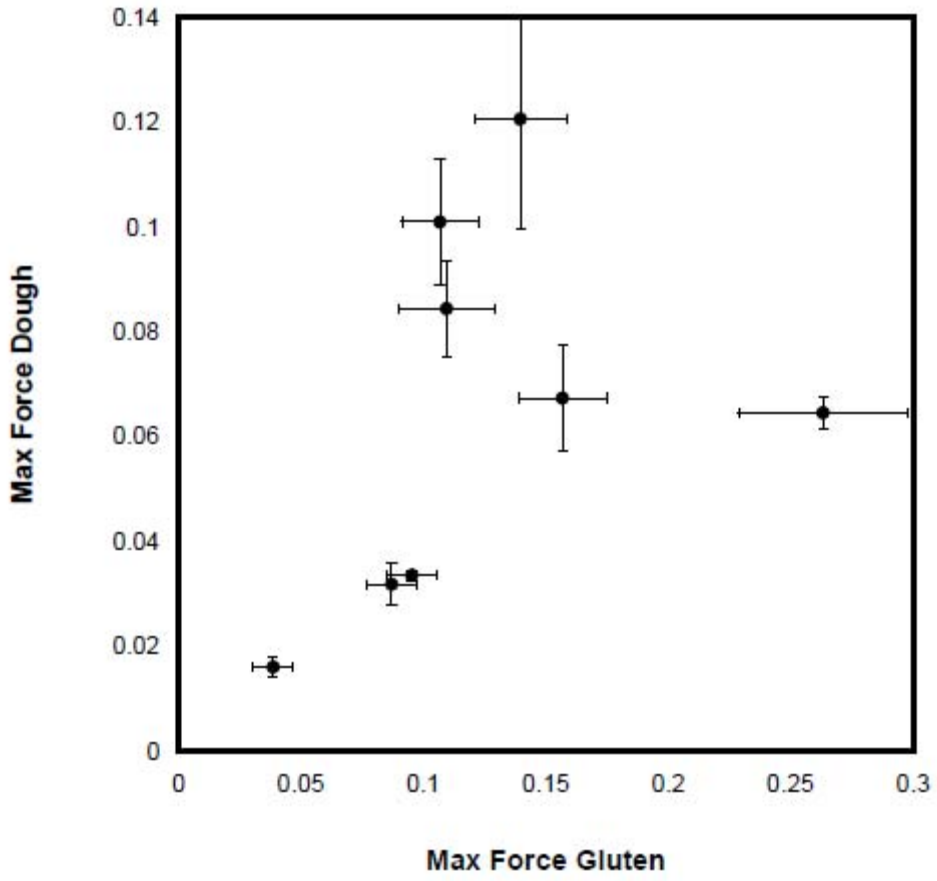

Figure 19 F_{max} for dough vs. with F_{max} for gluten of the same cultivars at 300% extension.

4.3 Conclusion

The TA-XT_{plus} stress relaxation assay used for gluten can also be appropriated for dough up to an extension of approximately 300% fold, and is suitable for studying dough relaxation behavior over longer time periods. As such it might be a useful tool for studying the effects of different protein compositions and strengths in a more complete system. Nonetheless humidity control would is necessary for studying dough behavior over longer time periods at the same hydration levels.

Further experimentation with a larger data set will be necessary to properly correlate gluten and dough strength, since the outliers cannot be readily explained at this time. Furthermore perhaps the tensile testing should be done from separately mixed dough samples, since the dough mixing most likely introduces further error. Nonetheless this dough tensile test would be a useful addition to the Mulvaney Lab repertoire, which is not currently equipped with a Kieffer extension rig, or a Brabender extensigraph.

Appendix

Maximum Force, Equilibrium Force, and Degree of Elasticity (DE) for select cultivars at different engineering strains alpha (L/Lo)

Cultivar	Maximum Force (N)	Equilibrium Force (N)	Degree of Elasticity (%)	Alpha (L/Lo)	Slope (N/cm)
Jagger	0.27	0.11	41.80	4	0.0127
	0.37	0.14	39.29	5	
	0.38	0.16	41.54	6	
	0.45	0.17	38.76	7	
Briggs	0.11	0.04	38.92	4	0.0187
	0.17	0.07	39.97	5	
	0.22	0.09	40.66	6	
	0.30	0.11	37.63	7	
McNeal	0.29	0.11	39.13	4	0.0158
	0.32	0.13	38.79	5	
	0.40	0.17	42.29	6	
	0.44	0.17	37.90	7	
Hollis	0.14	0.05	33.46	4	0.0098
	0.17	0.06	33.25	5	
	0.22	0.08	36.16	6	
	0.25	0.08	32.20	7	
Trego	0.26	0.10	37.75	4	0.0150
	0.31	0.12	37.60	5	
	0.32	0.13	41.22	6	
	0.42	0.16	37.87	7	
Blanca Grande	0.12	0.04	33.91	4	0.0106
	0.16	0.05	34.49	5	

	0.19	0.06	33.36	6	
	0.22	0.07	34.40	7	
Roane	0.10	0.04	43.63	4	0.0125
	0.16	0.05	32.63	5	
	0.20	0.08	37.35	6	
	0.22	0.08	36.83	7	
Stephens	0.04	0.01	31.92	4	0.0017
	0.05	0.01	29.83	5	
	0.04	0.01	32.29	6	
	0.07	0.02	29.61	7	
Eltan	0.09	0.03	36.36	4	0.0065
	0.09	0.03	37.21	5	
	0.13	0.05	39.26	6	
	0.14	0.05	36.98	7	

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